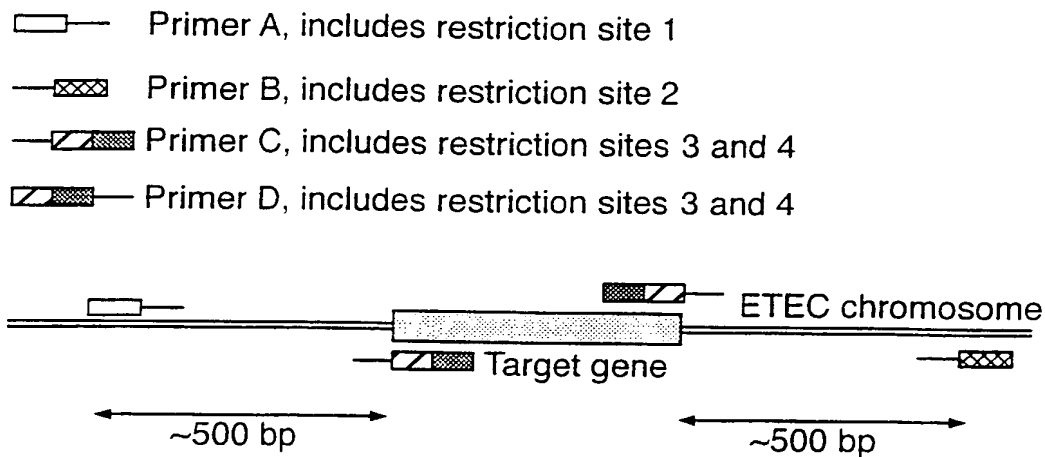


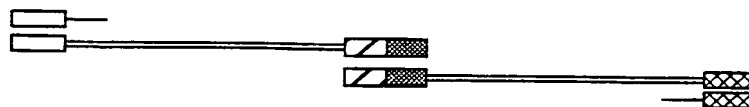
Fig.1.



PCR amplify target gene using primer pairs A/C and B/D



Gel purify PCR products



Amplify full length deletion cassette using equal amounts of PCR products 1 and 2 as template and primers A and B



Clone deletion cassette into pCVD442

Fig.2.

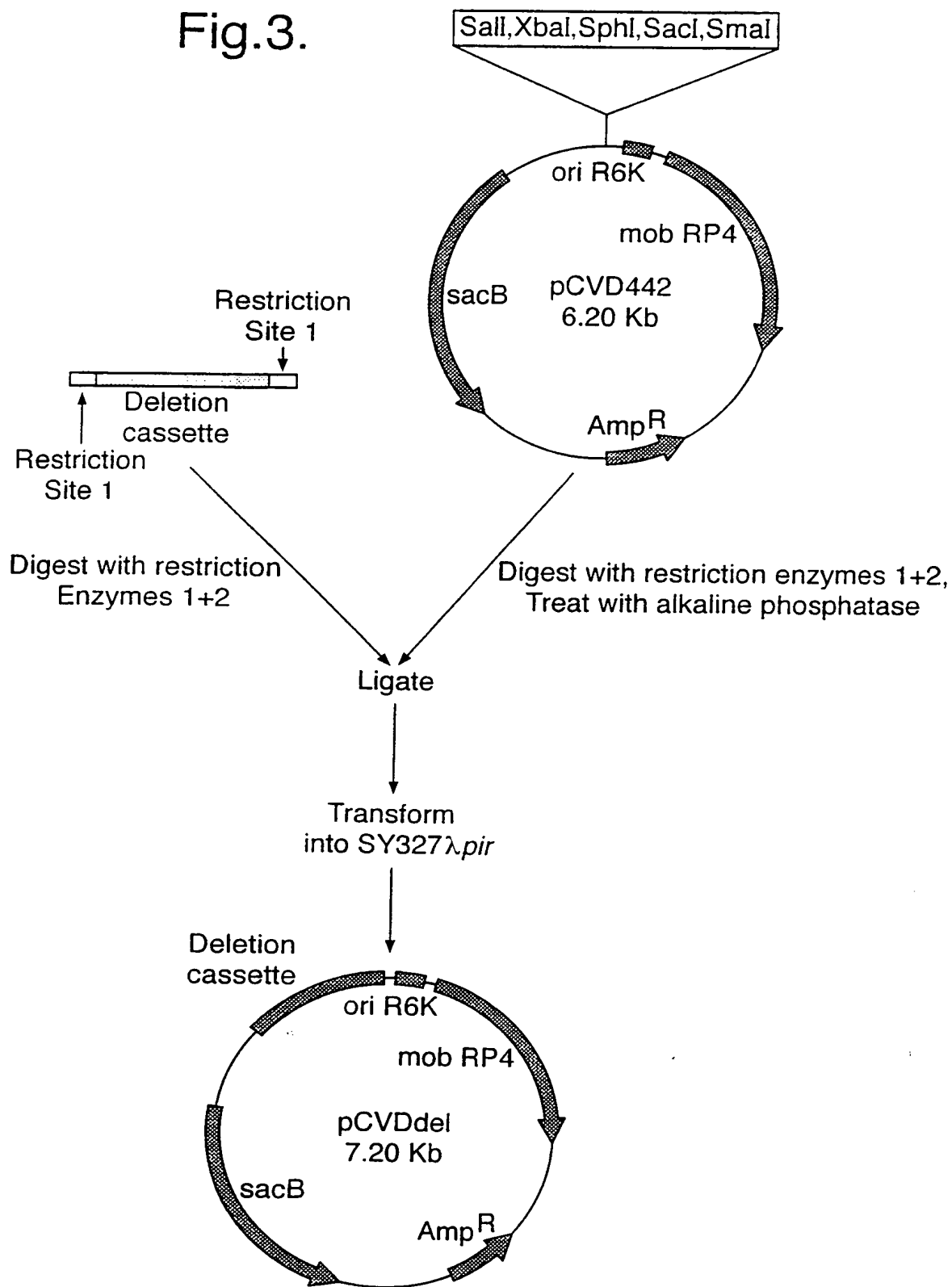
aroC	
w.t.	AAACACAACAATAACGGAGCGTGATG---TAAAAATGAATAAAACCGGATTG CG
deletion	<u>AAACACAACAATAACGGAGCCCTCGAGGCGATGCTGAATAAAATGAATAAAACCGGATTG CG</u>
htrA	
w.t.	TGTTAATCGAGAXTGAAATACATGAA---AGTAATCTCCCTCAACCCCTTCCT GAA
deletion	<u>TGTTAATCGAGAXTGAAATACCTCGAGTCTAGACTCCCTCAACCCCTTCCT GAA</u>
ompC	
w.t.	ATATAACAGAGGGTTAATAACATGAAA---CAGTTCTAA TCTCGATTGATATCGAAC
deletion	<u>ATATAACAGAGGGTTAATAACGCTAAGCCTCGAGTAA TCTCGATTGATATCGAAC</u>
ompF	
w.t.	AAACCATGAGGGTAATAAAATAATGATGAAGCGC---CCAGTTCTAA TAGCACACCTCTTTGTTA
deletion	<u>AAACCATGAGGGTAATAAAATAgagCTAAGCCTCGAGCAGTTCTAA TAGCACACCTCTTTGTTA</u>
ompR	
w.t.	CGAACCTTTGGGAGTACAAACAATGCAA---AAGCATGA GGCATTGCGCTTCTCGCCA
deletion	<u>CGAACCTTTGGGAGTACAAACAGCTAAGCGCATGCGA GGCATTGCGCTTCTCGCCA</u>

**Stop and start codons***Restriction enzyme sites introduced*Primer binding sitesLower case -- extra n.t added to primers to avoid primer dimer formation  
--- wild type gene

N.B. aroC deletion removes 16 n.t. 3' to the stop codon

3/6

Fig.3.



09646925.013101

4/6

Fig.4.

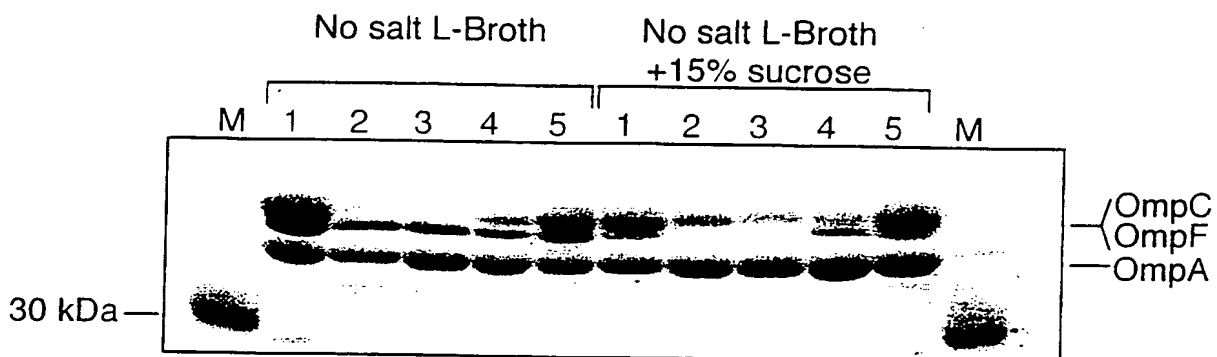


Fig.5.

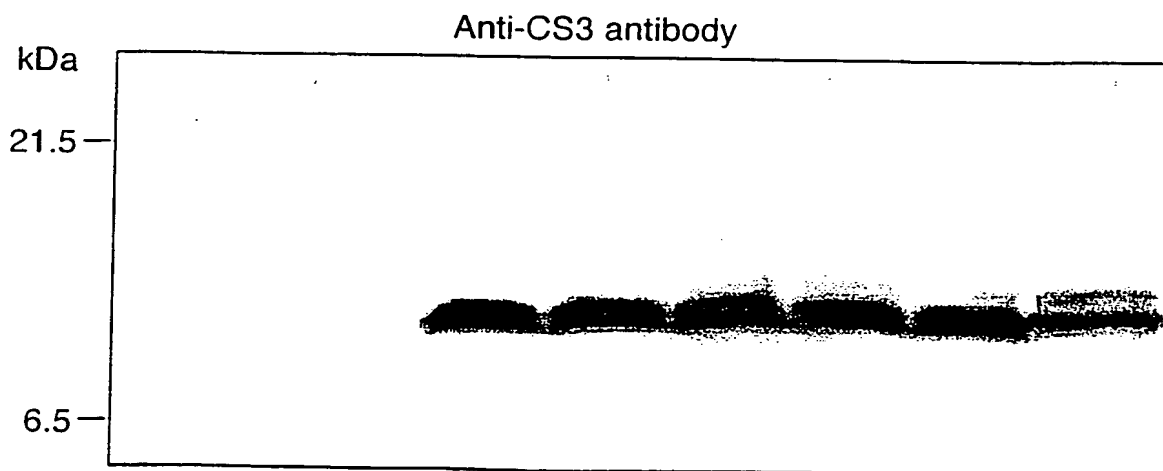
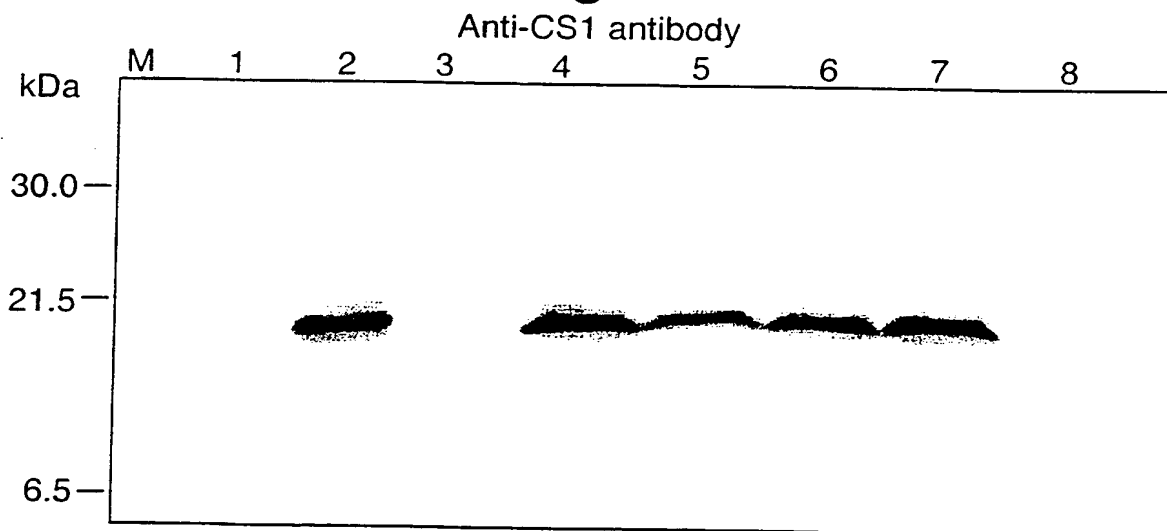
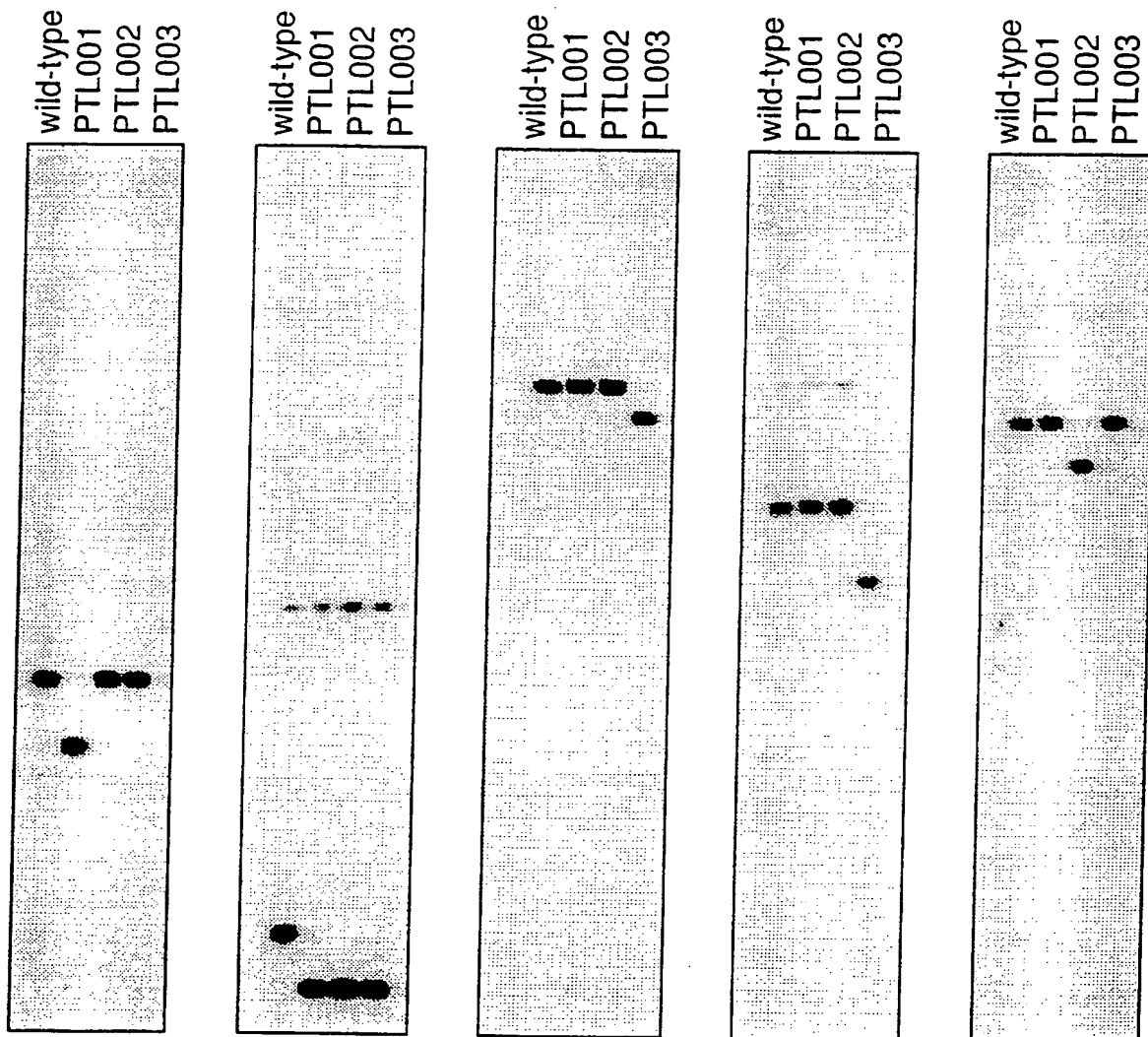


Fig.6.



6/6

Fig.7.

